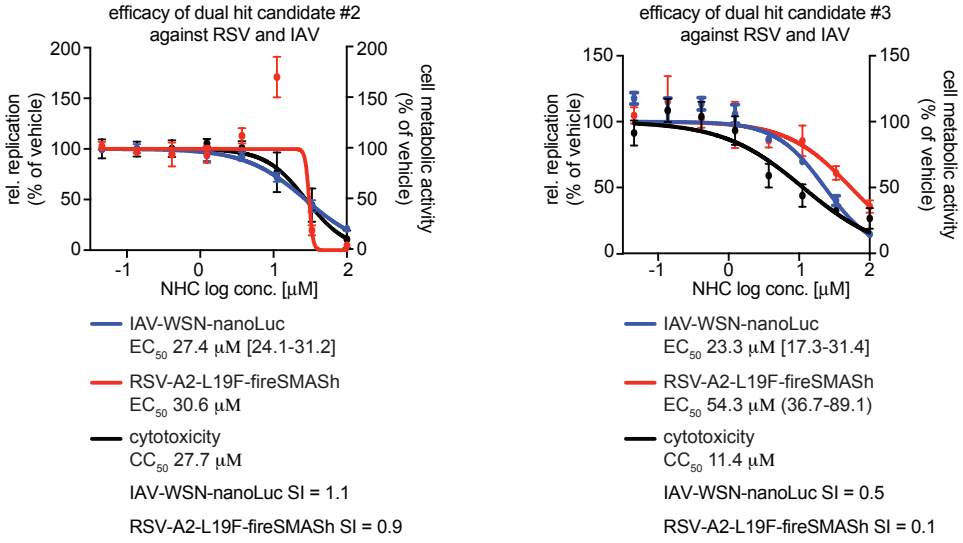


Fig. S1 Counterscreening of alternative dual IAV and RSV inhibitor hit candidates. Dose-response curves were generated for dual hit candidates #2 and #3 on IAV-WSN-infected MDCK and RSV-A2-L19F-infected HEp-2 cells. Cytotoxicity was quantified on MDCK cells using PrestoBlue cell viability assays after 48-hour exposure to the compounds. Values represent averages of three biological replicates ± SD. EC_{50} and CC_{50} values were determined through four-parameter variable slope regression modeling; 95% confidence intervals in brackets. Selectivity indices ($SI = CC_{50}/EC_{50}$) are shown.

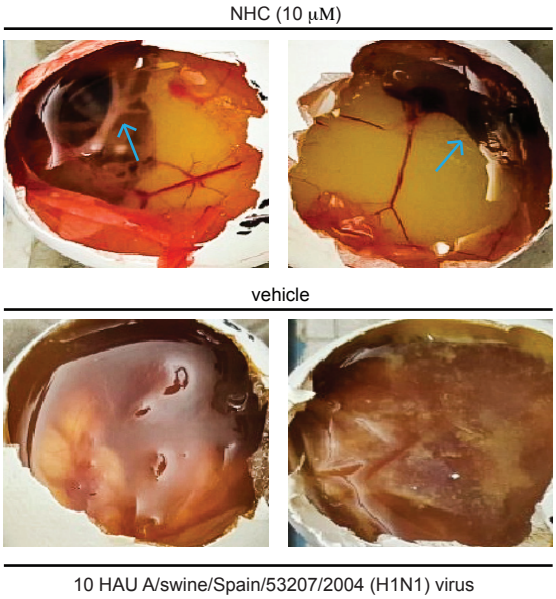


Fig. S2. Antiviral effect of NHC *in ovo*. Embryonated chicken eggs were infected with 10 HAU A/swine/Spain/53207/2004 virus and injected once with 10 μM NHC (final concentration) or vehicle volume equivalents. Arrows highlight the developing embryos in the treated eggs. Allantoic fluid was collected 48 hours post-infection, followed by opening of the eggs and photographic documentation.

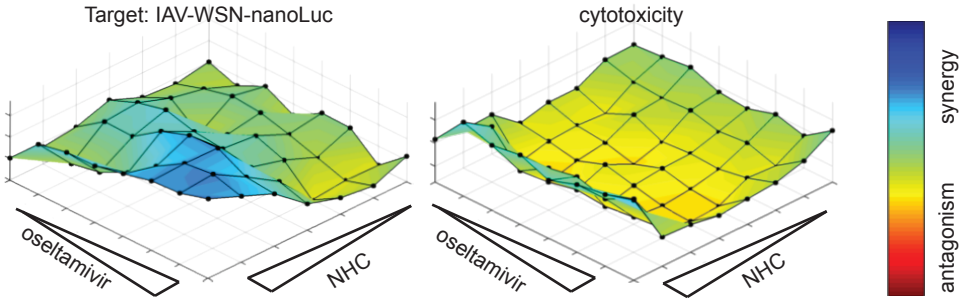


Fig. S3. Drug combination testing of SOC oseltamivir and NHC. MDCK cells in 96-well plates were treated with 49 dose combinations of NHC and oseltamivir (0.009 to 20 µM each in 3-fold dilutions) arranged in a checkerboard pattern. An additional row or column contained serial dilutions of each compound in isolation. Cells were infected with IAV-WSN-nanoLuc and reporter activity measured 48 hours post-infection. Data sets representing five biological repeats were probed for areas of synergy using the HAS model in the Combenefit software package (z-axis: % inhibition; blue: areas of potential synergy; areas of antagonism would appear red). Relative synergy score syn_{max} 27. Cytotoxicity of the equivalent compound combinations was assessed in uninfected MDCK cells through PrestoBlue viability testing after 48-hour exposure. Data sets representing three biological repeats were probed for areas of synergy of toxicity as before. None of the compound combinations significantly enhanced cytotoxicity (syn_{max} 14).

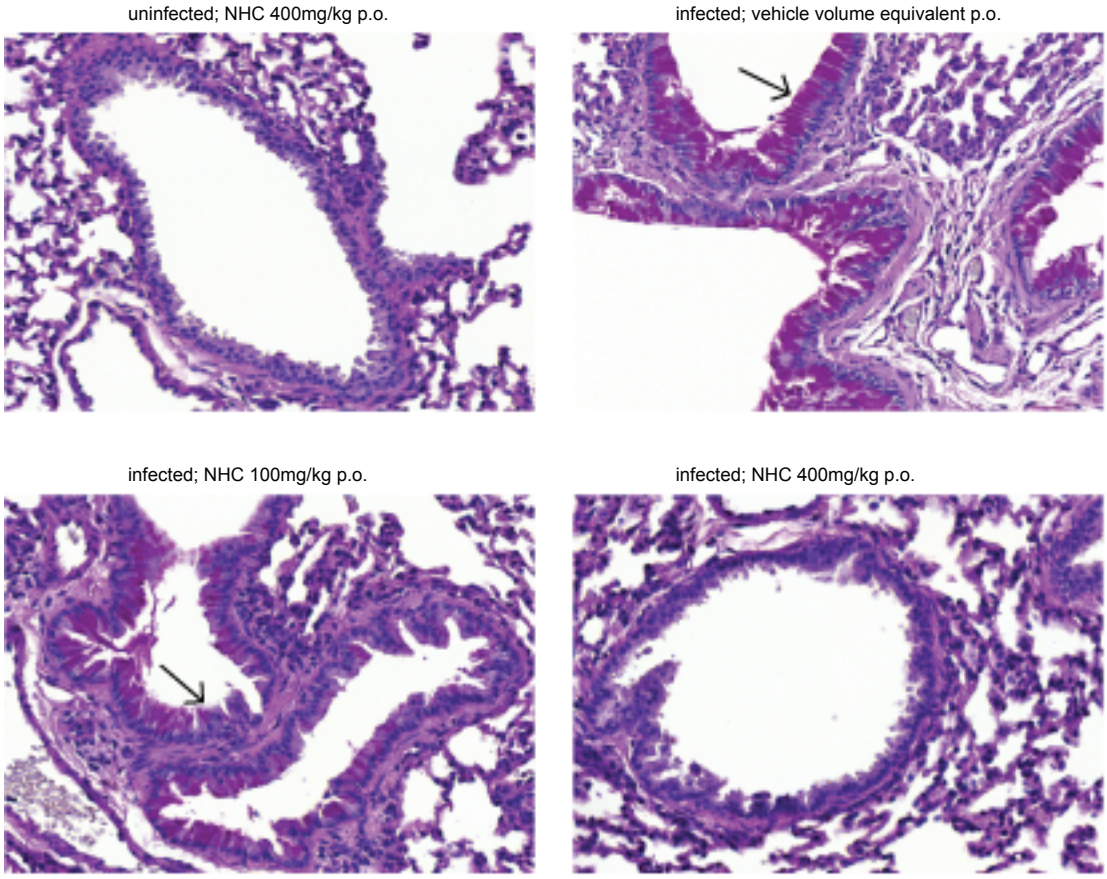


Fig. S4 Mucin expression in BALB/cJ mice lung after RSV infection. 5 µm tissue sections were prepared from uninfected and RSV-A2-L19F infected animals eight days post-infection, paraformaldehyde-fixed and paraffin-embedded. Oral NHC treatment was initiated prophylactically two hours pre-infection and continued b.i.d. Tissue sections were stained with periodic acid-Schiff (PAS). Extensive mucin deposits (arrow) were detected in lungs of vehicle-treated, infected animals. To a smaller extent, mucin was present in animals receiving doses of 100 mg/kg p.o. NHC, but absent from lungs of animals dosed at the 400 mg/kg p.o. NHC level.

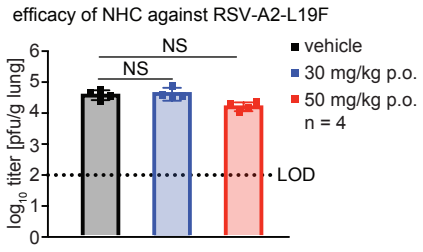


Fig. S5 Oral efficacy of NHC against RSV at low dose concentrations. BALB/cJ mice were infected intranasally with 1×10^5 pfu of recRSV-A2-L19F, lungs harvested four days p.i., and viral titers determined through immunoplaque assays. Oral NHC treatment was initiated prophylactically and continued b.i.d. Symbols represent individual animals of each group, columns show means ± SD; 1-way ANOVA with Tukey's multiple comparisons test; NS not significant.

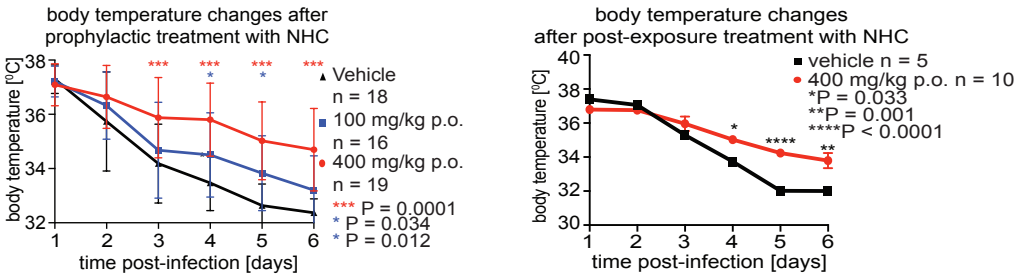
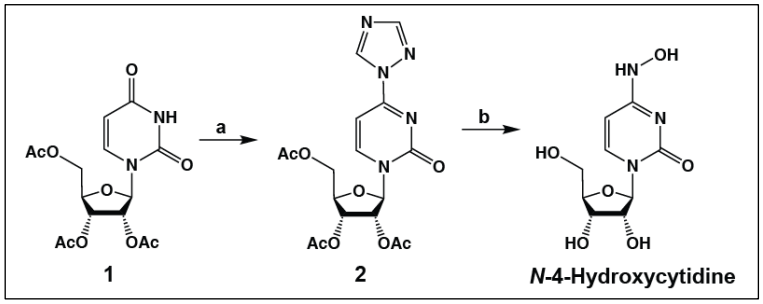


Fig. S6 Clinical signs in IAV-infected NHC-treated mice. BALB/cJ were infected intranasally with 1,000 pfu of IAV-PR8. Mice body temperature changes after prophylactic (left) or post-exposure (right) oral NHC treatment b.i.d for six days. Post-exposure NHC treatment was initiated six hours post-infection days. Animals were monitored daily and changes are expressed in absolute measurements. Analysis with 2-way ANOVA and Dunnett's multiple comparisons test for prophylactic treatment and with 2-way ANOVA Sidak's multiple comparisons test for post-exposure treatment.

Supplemental text S1: Chemical synthesis of NHC

Synthetic scheme and purity measurements of NHC. Reagents and Conditions: a) 1.6 eq POCl3, 7.2



eq triazole, 8 eq Et3N, MeCN, RT, 2 h, 82% yield; b) 10 eq 50% aqueous hydroxylamine, iPrOH, RT, 18 h, 87 % yield. (2R,3R,4R)-2-(acetoxymethyl)-5-(2-oxo-4-(1H-1,2,4-trizol-1-yl)pyrimidin-1 (2H)-yl)tetrahydrofuran-3,4-diyl diacetate, **2**: A 3 L 3-neck flask equipped with an overhead stirrer, temperature probe and addition funnel was charged with 1,2,4-triazole (269 g, 3.89 mol) in 1200 mL of acetonitrile and the mixture was stirred at RT for 10 minutes. The mixture was cooled using an ice bath and phosphorous oxychloride (81 ml, 0.86mol) was added at less than 20°C. After the addition was complete, the mixture was stirred at 5°C for 20 minutes and triethylamine (602 mL, 4.32 mol) was slowly added at less than 20°C. The mixture was stirred at 5°C for 30 minutes and then an acetonitrile (500 mL) solution of 2',3',5'-tri-O-acetoxyuridine (200 g, 0.54 mol) was added over 10 minutes. The resulting slurry was stirred at RT until the starting material was consumed, (2 hrs). The reaction was quenched by the addition of 100 mL of water, stirred for 30 minutes and concentrated under reduced pressure to yield a pale yellow solid. The residue was partitioned between 1500 mL of dichloromethane and 500 mL of saturated bicarbonate solution. The aqueous layer was extracted with dichloromethane (2×250 mL); the combined organics were washed with 500 mL of bicarbonate, 500 mL of brine, dried over sodium sulfate, filtered and concentrated under reduced pressure at 45 °C to ~50 % volume. Ethyl acetate (1,500 mL) was added to the solution and then concentrated to ~500 mL under reduced pressure at 45°C. The resulting slurry was stirred at 45°C for ~1 hr. After cooling to RT, the solid was collected by vacuum filtration, washed with ethyl acetate (2×100 mL), and then ether (2×200 mL) and dried in vacuo to a constant weight to yield (2R,3R,4R)-2-(acetoxymethyl)-5-(2-oxo-4-(1H-1,2,4-triazol-

1-yl)pyrimidin-1(2H)-yl)tetrahydrofuran-3,4-diyl diacetate (187 g, 444 mmol, 82% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.44 (s, 1H), 8.46 (d, J = 7.3 Hz, 1H), 8.41 (s, 1H), 7.07 (d, J = 7.2 Hz, 1H), 6.00 (d, J = 3.6 Hz, 1H), 5.54 (dd, J = 6.0, 3.6 Hz, 1H), 5.36 (t, J = 6.3 Hz, 1H), 4.37 (ddd, J = 12.8, 7.3, 3.1 Hz, 2H), 4.27 (dd, J = 12.3, 5.6 Hz, 1H), 2.08 (d, J = 0.5 Hz, 3H), 2.05 (d, J = 0.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d6) δ 170.52, 169.72, 159.60, 154.67, 153.89, 149.83, 144.30, 109.99, 95.12, 91.15, 79.92, 73.38, 69.85, 63.15, 21.01, 20.71. HRMS ESI: m/z [M+H]+ calcd. for C17H20N5O8: 422.13119, found 422.13048 N-4-Hydroxycytidine: A 500 mL RBF was charged with [(2R,3R)-3,4-diacetoxy-5-[2-oxo-4-(1,2,4-triazol-1-yl)pyrimidin-1-yl]tetrahydrofuran-2-yl]methyl acetate (18.7 g, 44.38 mmol), isopropyl alcohol (100 mL) and a 50% aqueous hydroxylamine solution (27.2 mL, 443.8 mmol) was added at RT. The clear colorless solution was stirred at RT for 18 hrs; white precipitate formed during this time. The mixture was cooled in an ice bath, diluted with an additional 100 mL of isopropyl alcohol and stirred at 0°C for 1 hr. The precipitate was collected by filtration, washed with cold isopropanol, ether and dried in vacuo to yield 10 g of N-4-Hydroxycytidine as a crystalline, white solid (39 mmol, 87% yield). The material was recrystallized by dissolving the solid (5.0 g, 19.29 mmol) in 50 mL of hot 95% EtOH, filtering to remove any residual solid and allowing the supernatant to slowly cool to RT. The flask was placed in the freezer for at 1hr, then the solid was collected by filtration and washed with a small portion of cold EtOH (2×20 mL), ether (2×30 mL) and dried in vacuo to yield (4.5 g, 13.39 mmol, 90% yield) as a white solid. ^{1}H NMR (400 MHz, methanol-d4) δ 7.16 (d, J = 8.2 Hz, 1H), 5.86 (d, J = 5.6 Hz, 1H), 5.59 (d, J = 8.3 Hz, 1H), 4.18 - 4.07 (m, 2H), 3.93 (q, J = 3.3 Hz, 1H), 3.77 (dd, J = 12.1, 2.9 Hz, 1H), 3.68 (dd, J = 12.1, 3.4 Hz, 1H). ^{13}C NMR (101 MHz, methanol-d4) δ 150.60, 145.14, 130.85, 98.23, 88.08, 84.48, 73.05, 70.20, 61.26. LCMS: m/z 260.1 [M+H]+. HRMS ESI: m/z [M+H]+ calcd. for C9H14N3O6: 260.08826, found 260.08839. Anal. calcd for C9H13N3O6 (included 1.91% water as determined by coulometric Karl Fischer titration): C, 40.91; H, 5.18; 15.91. Found: C, 40.55; H, 5.35; N, 15.65.